MIqPCR: *Minimum Information about a Quantitative Polymerase Chain Reaction experiment*

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RDML-Consortium

This module identifies the minimum information required to report the use of quantitative Polymerase Chain Reaction (qPCR) assay in a manner sufficient to support the unambiguous interpretation of the results presented and the potential corroboration of the conclusions drawn.

Introduction

Quantitative polymerase chain reaction (qPCR) assays measure the copies of a specific DNA target in a sample as that sample is repeatedly passed through the polymerase chain reaction. Special qPCR machines are required to quantify the amplification products at each step of the cycle. MIqPCR describes the minimal information needed to allow effective interpretation and reanalysis of the data generated by such assays. For further background on this and related projects appropriate for qPCR, please visit the website† (http://www.rdml.org).

The following section, detailing the reporting requirements for the use of qPCR, is subdivided as follows:

1. Administrative information
2. Sample annotation
3. Target annotation
4. Thermal Cycling Conditions
5. Run data
6. Software requirements

Reporting requirement for Quantitative PCR Assays

1. Administrative information

a) Experiment description
- Experiment description
- Responsible person and contact details

2. Sample annotation

a) Sample description
- Sample ID
- Sample description
- cDNA synthesis method and DNAse treatment (cDNA samples only)
- Template quantity (standard and optical calibrator samples only)

b) Sample role in qPCR assay
- Sample type
- Inter run calibrator (true or false)
- Calibrator sample (true or false)

3. Target annotation

a) Target description
- Target ID

- Sequence of primers *OR* commercial assay description

b) Target role in qPCR assay
- Target type

4. Thermal Cycling Conditions Information

a) PCR program
- Complete description of the cycling conditions

5. Run data

a) Instrument information
- Plate format
- Instrument description
- Software description and version

b) Information required for each well
- Well ID
- Sample ID
- Target ID
- Amplification curve fluorescence values for each data point
- Melting curve fluorescence values for each data point
- Quantification Cycle

6. Software requirements

a) RDML-Support
- Software solutions, including databases, must support the import and export of RDML files.
- qPCR machines must allow the export of raw data for the amplification as well as for melting curves.

Summary

These minimum reporting requirements for quantitative PCR assays specify that a significant degree of detail be captured, for sample and target description, PCR amplification and data capture and analysis, and that in addition the raw and processed data are also made available. However, it is clear that providing the information required by this document will enable the effective interpretation and assessment of qPCR data and protocols and potentially, support experimental corroboration. Much of the information required herein may already be stored in an electronic format, or exportable from the instrument; we anticipate further automation of this process.

These guidelines will evolve. To contribute, or to track the process to remain 'MIqPCR-compliant', browse to the website at http://www.rdml.org/

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Experiment description: A sentence or short phrase describing the context of the experiment, or a stable pointer to contextualizing information, such as a database record or a publication.

Responsible person and contact details: Contact details of the experiment supervisor, this includes the name and email address, and name and postal address of the institute

Sample ID: Unique identifier for a given sample, to be used in the run data as reference for the sample.

Sample description: A sentence or short phrase describing the sample.

cDNA synthesis method: Enzyme and priming method used for cDNA synthesis. Possible priming methods are: *oligo-dt*, *random*, *target specific* or *any combination of the above*.

DNase treatment: Boolean value to indicate whether nucleic acids have been treated or not with DNase. Possible values are *true* and *false*.

Template quantity: Nucleic acid quantity of a standard sample

Sample type: Describes the type of sample. Possible values are : *unknown*, *no template control*, *no amplification control*, *standard* or *optical calibrator*.

Inter-run calibrator: Boolean value to indicate whether or not a sample can be used to detect and correct inter-run differences. Possible values are *true* and *false*.

Calibrator sample : Indicates if a sample is used as a reference to rescale all relative measurements to (e.g. untreated control). Possible values are *true* and *false*.

Target ID: Unique identifier for a given target, to be used in the run data as a reference to the detailed target information.

Sequence of primers: Nucleotide sequence of the PCR primers. The amplicon sequence and optional 3'- and 5'-tags can also be mentioned.

Commercial assay description: Short description of the commercial assay used for PCR amplification, includes at least company name and order number.

Target type: Describes the use of a target. Possible values are: *reference* and *target of interest*.

PCR program: Describes the thermal profile used for the PCR amplification and detection of accumulating amplicons. The program consist of one or multiple steps, each with it own characteristic information (e.g. duration, temperature, type of measurement, a loop with number of repeats, etc.)

PCR format: Description of the run format used in the experiment (for example 96-well plate A1-H12, or 32-well rotor 1-32).

Instrument description: Name of instrument and vendor

Software description and version: Contains the name and version of the software used for the analysis. The methods used for determining the background and calculating the quantification cycle can be included as well.

Well ID: Identifier for a given well. Format is free, but should preferably reflect the run format.

Amplification curve fluorescence values: Contains the amplification fluorescence data for a given amplicon. The data is organized per sample and per amplicon with a corresponding cycle number for each data point.

Melting curve fluorescence values: Contains the fluorescence data of the melting curve for a given amplicon. The data is organized per sample and per amplicon with a corresponding temperature for each data point.

Quantification Cycle (Cq): Universal name for the fractional PCR cycle at which the target is quantified in a given sample. Depending on the real-time instrument, either crossing point (Cp), cycle threshold (Ct) or a take-off point (Top) are used to refer to the same value (Cq).

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